コフキタケの担胞子の発芽

青　島　清　雄[1)]

緒　言

担子菌類に属する木材腐朽菌の担胞子の発芽は胞子が子実層から落下後，適当な培養基上で適当な温度を与えた場合には 48 時間以内に容易に発芽する。この知見は内外多数の研究者により，多数の資料に基いて実験的に確認されている。ただコフキタケ *Elfvingia applanata* (PERS.) KARST. は WHITE (25) の報告以来，1つの例外と認められてきたのである。 彼によれば本菌の担胞子は発芽がきわめて不規則で，発芽する場合には培養液或いは蒸溜水中で接種後 48 時間以内に発芽するが，発芽率はきわめて低く，わずかに 1～2 パーセントを算するに過ぎない。さらに担胞子が落下後 6 カ月経過すると全く発芽の力を失うという。

本菌が熱帯から寒帯に広く分布し，広葉樹の生立木や土木用材に大きな被害を与えていることから彼の結論は筆者に1つの疑問を与えたのであるが，筆者は WHITE の実験の追試を行つて全く新たな知見を得ることが出来た。

この研究に対して御指導，御鞭撻を賜つた林業試験場今関六也保護部長，永井行夫菌類研究室長，長尾研究所小南清所長に深く謝意を捧げる。また，アメリカのエール大学 J. S. BOYCE 教授はこの原稿に目を通され，貴重な助言を賜つた。ここに深く御礼申上げる。

寒天培養基および液体培養基中での担胞子の発芽

方法：担胞子の採取はまず本菌の子実体が春になつて胞子を落下撒布させはじめるのを見定めてから， 子実層の下にパラフィン紙をおいて，24 時間の間に落下附着したものを1集団として用いた。担胞子の発芽検査には，このパラフィン紙に附着した胞子を殺菌蒸溜水に懸濁させて寒天培養基上にぬりつけた。液体培養基の場合はパラフィン紙に附着したものを直に用いた。担胞子を1つづつ取出すには顕微鏡下で，寒天培養基上に固定した胞子を白金の輪で寒天とともに切取つた。

実験（1）：6種の天然および人工寒天培養基[2)] を用いて新鮮なコフキタケの担胞子の発芽を観察したが，48 時間後にはそれぞれ 20°C，25°C および 30°C では観察個体数 1000 以上

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2）馬鈴薯煎汁培養基（常法）；前者に 0.2% のタンニン酸を混入したもの；麦芽煎汁培養基 （常法）；斎藤氏処方醤油培養基；ツアペツク氏処方培養基；蒸溜水のみ

のうちで1つも発芽したものはなかつた。6カ月後に同一の胞子と改めて新鮮な胞子の発芽を検したが，全く同様の結果が得られた。

次に馬鈴薯煎汁（2％の葡萄糖を加える）と斎藤氏処方醤油培養基の pH を2から 9の8段階に変化させてそれぞれ Van Thiegem のセルを用いて発芽を調べたが，それぞれの観察個体数 1000 以上のうちで，1つも発芽したものはなかつた。前の実験と同じように繰返し行つた実験でも同様に発芽したものは見られなかつた。

実験（2）：前の実験では雑菌の混入のために長期にわたつて胞子の発芽を検する訳にはいかないので，担胞子を1つづつ1本1本の試験管（馬鈴薯煎汁寒天培養基が入つている）に取出して，長期間観察をつづけた。結果は Table 1. に示すごとく，1週間以内に発芽してきたのはわずかに1パーセント以下であるが，4カ月にわたつて不規則な発芽を示した。発芽した胞子からの菌糸は Fig. 1 に示すごとく，最初は薄い蜘蛛巣状であるが，漸次皮革状の菌叢となつてくる。

実験（3）：前の実験よりもさらに多数の胞子の発芽を定期的に検査した。冬季は 25°C の定温においたものの発芽を Fig. 2 に，冬季も室温においたものの発芽を Fig. 3 に示す。この2つの図からコフキタケの担胞子は休眠期を待つていることが明らかにされた。そして寒天培養基上にまいた胞子も5カ月後にわずか 10 パーセント程度が発芽するに過ぎない。

実験（4）：前の実験に使つた子実体の生じている樹木と約200米離れた樹木の切株に別の子実体が生じていたので，この子実体からとつた担胞子の発芽を検査した。結果は Fig. 4 に示すごとく，前の実験と大差がないことが知られる。

実験（5）：同一の子実体に生じた担胞子で室温下に1カ年保存したものと新鮮な胞子の発芽をそれぞれ同時に検査したのが Table 2. であるが，この表から室温下で1カ年保存した胞子は全く発芽力を失うことが知られる。

担胞子の発芽と温度との関係

材料および方法は前の実験と同様であるが，それぞれ 20°C，23°C，25°C，28°C および 33C° で発芽を検査したのが Table 3. である。本菌の担胞子は 20～33°C で発芽可能で，23～28°C で最も良く発芽する。しかし 20°C 以上，あるいは 33°C 以上で発芽するか否かは不明である。

高温処理による休眠打破

実験（1）：乾燥状態で 50°C，60°C および 70°C にそれぞれ 5，10，30，60 および 240 分間胞子を触れさせて発芽を検討した。水中では 40°C，45°C，50°C にそれぞれ 10，30，60 および 240 分間触れさせて検査したが，乾燥状態でも水中でも，培養基に接種後 48 時間以内には発芽したものは全くみられなかつた。

実験（2）：前の実験よりも胞子の発芽をさらに長期にわたつて観察した。結果は Fig. 5 および Fig. 6 に示す通りである。24 時間処理の場合は 40°C が最も効果があり，35°C がこれにつぎ，30°C は全く効果がない。48 時間処理の場合は 35°C，30°C および 40°C の順に効果がある。

論議および結論

材質腐朽性の担子菌は寒天培養基や液体培養基中でそれぞれの適温のもとでは 48 時間以内に発芽するのが通則である (1, 3, 10, 13, 18, 19, 20, 22, 24)。 本菌については White (25) の報告があるが， 彼によると培養基に接種してから 48 時間以内に発芽可能のものは発芽を完了するという。この結果は Hubert (15)，Bavendamm (2)， Cartwright および Findlay (6) によつて認められてきた。

筆者の実験によれば本菌の担胞子は培養基や温度の如何に関せず，48 時間以内には全く発芽しない。さらにこの菌の担子胞子は短いもので 1 週間，長いもので 1 カ年の休眠期を有することが実験的に確められた。 筆者の実験と White の実験結果とは全く異つているが， この違いは説明が出来ない。しかし，筆者の実験では発芽数——時間曲線は正規の S 字曲線が描かれている。

東京附近で 6 月に形成された本菌の担胞子は年内にその 10 パーセント程度が発芽するに過ぎず，翌春再び発芽を開始する。そして発芽はその年の秋までつづく。したがつて，自然界では本菌の担胞子は越冬の可能性が充分あると思われる。

White (l. c.) は本菌の担胞子の発芽は 1 ～ 2 パーセントに過ぎないと述べているが，筆者の実験によれば，もしも 1 カ年以上にわたつて観察をつづけるとその 50% 以上が発芽することを確めた。

本菌の担胞子は 20～33°C で発芽可能で，発芽の適温は 23～28°C である。

高温処理による胞子の休眠打破は Dodge (9)，Shear および Dodge (23) および Goddard (12) によつて子嚢菌の *Ascobolus* や *Neurospora* について得られた効果とは異にし，35°C，30°C および 40°C で 48 時間，40°C および 35°C で 24 時間の処理が効果があるこ

とが確められた。この場合，発芽促進の効果のみで最後の発芽率には何の影響もない。

担子菌の中で，その担胞子が休眠期を持つていることや，高温処理が休眠打破に効果があるということはきわめて特異的で，いまだ報告された例がないが，これは本菌の担胞子の膜が厚く，しかも特殊な構造を持つているために起る現象であろう。

なお，筆者はマンネンタケ（*Ganoderma lucidum*）の担胞子の発芽がコフキタケの担胞子と同じような性質を持つていることを確めたが，一般にマンネンタケ族（*Ganodermoideae*）の担胞子は発芽に際してはコフキタケとほとんど同様な経過をたどるものと推定される。

Neurospora の子囊胞子の高温処理による休眠打破はガスの透過性が問題ではなく，carboxylase の賦活に基くものとされているが，担子菌に属するコフキタケの担胞子では実験的に確めてはいないが，休眠打破が急速に行われ難い点から前に記した胞子の厚く，複雑な膜によるガスおよび水分の透過性が減少しているためとみるのが至当のようである。また，高等植物硬実種子のように酸による処理やアルカリによる処理は筆者が現在までに得た結果からは全く効果がない。

Kiyowo Aoshima: Germination of the basidiospores of *Elfvingia applanata* (Pers.) Karsten (*Fomes applanatus*).

Contents

Introduction

Elfvingia applanata (Pers.) Karsten is known among mycologists, pathologists and foresters as one of the commonest wood-rotting fungi. It is widely distributed from the tropic to the subarctic forests in the world causing a white rot in various species of broad-leaved and also, though less frequently, in coniferous trees.

A large number of reports have been published concerning the morphological and taxonomic, cultural, physiological and pathological problems of this fungus, such as White (1920), Fritz (1923), Humphrey and Leus (1931), Yamano (1931), W. G. Campbell (1932), Humphrey and Siggers (1933), W. A. Campbell (1938), Davidson, Campbell and Vaughn (1942) and Nobles (1948).

On the germination of the basidiospores, however, a paper by White (1920) is the only work, so far as the writer is aware, up to the present.

The writer has undertaken from May, 1950, some observations and experiments on the germination of basidiospores and in this paper some results concerning their dormancy will be given.

The writer wishes to express his sincere thanks to Mr. R. Imazeki and Mr. Y. Nagai of the Government Forest Experiment Station, Meguro, Tokyo and Mr. K. Kominami of the Nagao Institute, Kitashinagawa, Tokyo for their helpful

abvice and encouragement during the investigation. He also express his cordial appreciations to Dr. J. S. BOYCE of the Yale University, New Haven, Connecticut, U. S. A. for his kind suggestions and criticism of the manuscript. To Mr. T. KOBAYASHI the writer indebted for his assistance in the laboratory work.

Germination of the basidiospores on agar and in liquid media

Experiment (1)

WHITE (l. c.) reported in his biological study on *Elfvingia applanata* that the germination of basidiospores of this fungus was very erratic and when it did occur, gemination took place within 48 hours after the basidiospores had been placed on agar media, in water or other liquid media, but the germination percentage thus obtained was very small, being about 1.5 per cent. The writer has also undertaken the same observations using various agar and liquid media.

Materials: A fruiting-body of *Elfvingia applanata* (about 15 cm long, 5 cm wide and 3 cm thick) on an old living trunk of oak tree (*Quercus myrsinaefolia*) growing at Meguro, Tokyo, was found. On 2 nd June, 1950, abundant brown powder was observed on the bark of the host tree just below the fruiting-body and on the upper surface of the sporophore. Brown masses were also present on the underside or pore surface of the sporophore. The brown powder was examined under the microscope to determine that it was composed of masses of basidiospores. Immediately after the spore-discharge was examined, a piece of paraffin paper was fixed just beneath the pore-surface of the same sporophore and the sporophore was covered with a large paper bag in order to prevent entrance of other microorganisms and insects. Fifteen hours later, at night, the paper bag kept outside the fruiting-body and a piece of paraffin paper beneath the pore sprface were removed. Numerous basidiospores were deposited on the piece of paraffin paper about 0.2 mm deep. In this way the writer obtained easily the relatively pure mass of basidiospores.

Method: In a test-tube (20 cm high and 1.5 cm in diameter) containing 10 cc of distilled water, a mass of basidiospores was put in with a platinum needle and stirred, thus a spore suspension of basidiospores was obtained which had some shade of brown. In Petri dishes (7 cm in diameter) very clear potato-glucose agar was poured 1—2 mm deep, and some drops of the spore suspension were spread over it. Agar media used in this experiment were the following six: distilled water agar[1], potato-glucose agar[2], SAITO's soy agar[3], beech-wood extract agar[4], potato-glucose agar containing 0.2 per cent of tannic acid[5] and CZAPEK's synthetic agar[6]. After drops of water on the surface of the agar were

1). 2% distilled water solution of agar 2). Potato-decoction (200 gr of potato in 1000 cc of distilled water), 1000 cc; glucose, 20 gr 3). Onion decoction (100 gr of onion in 100 cc of water), 100 cc; Soy, 500 cc; water, 850 cc; agar, 20 gr 4). Saw-dust of sap-wood of beech, 30 gr; distilled water, 100 cc; agar, 20 gr 5). Potato-decoction, 1000 cc; glucose, 20 gr; tannic acid, 2 gr; agar, 20 gr 6). $MgSO_4\ 7H_2O$, 0.5 gr; K_2HPO_4, 1 gr; KCl, 0.5 gr; $NaNO_3$, 2 gr; sucrose, 30 gr; $FeSO_4$, 0.01 gr; water, 1000 cc; agar, 20 gr

dried, the Petri dishes were transferred to incubators kept at the constant temperatures of 20°C, 25°C and 30°C, respectively. After 48 hours incubation, microscopic observations were carried out on germination of the spores.

Results: It was found that none of the basidiospores, among more than one thousand observed, germinated in any media and at any temperatures mentioned above. The same results were obtained on 12 th October, 1951 using the same basidiospores and basidiospores obtained from another fruiting-body on 24 th August, 1951.

Next, observations were performed using the liquid media of potato extract adding 2 per cent glucose, and SAITO's soy extract varying the pH value, with KOH and H_3PO_4, from 2 to 9 at intervals of about 1. After inoculation followed by incubation of the VAN THIEGEM's cells for 48 hours at 20°C, 25°C and 30°C, a microscopical examination was made. Not one of the more than one thousand basidiospores observed had germinated. On 12 th October, reexamination was carried out and the same results were obtained.

It is shown by the writer's four series of experimets shown above that the basidiospores of *Elfvingia applanata* do not germinate on the agar and in the liquid media 48 hours after inoculation at their favourable temperatures.[1)]

Experiment (2)

In this experiment the basidiospores of this fungus were observed for longer periods of time than 48 hours. A single basidiospore isolated on the potato-glucose agar in a test tube was observed in order to avoid contamination by other microorganisms in the cource of the observation for longer periods of time. The materials used in the present experiment were quite the same as in the preceding ones.

Method: In Petri dishes (7 cm in diameter) very clear potato-glucose agar was poured 1—2 mm deep. After drops of water on the surface of each Petri dish containing the agar medium, side by side in the same direction. With a platinum needle a drop of spore suspension was taken and streaked from one end to the other of the pencil line on the agar medium. In order to vaporize the water-drops containing the basidiospores which were put on the agar medium, the Petri dishes were placed in a dessicator at room temperature for 20—30 minutes. Then single basidiospores were selected using the low power of a microscope, picked up in a small block of agar by means of a platinum wire ring, and transferred to a test tube containing potato-glucose agar. Care was excercised to avoid polysporous inoculum and the writer obtained 150 single basidiospores each in a test tube. Isolation of single basidiospores was carried out on 3 rd June, 1950 and observations were made to determine whether the mycelium originating from single basidiospores of this fungus were visible or not at irregular intervals from June to the end of September, 1950.

1). Temperatures in relation to germination will be given in the latter experiment of this paper.

Results: Table 1 shows the number of germinated basidiospores from which the mycelium was just visible to the naked eye.

Table 1. Number of germinated basidiospores among 150 spores during 4 months periods.

Date examined		Number of germinated basidiospores	Total
June	10	1	1
June	20	0	1
July	1	0	1
August	30	1	2
Sept.	25	8	10

Fig. 1. Mycelia of *Elfvingia applanata* resulting from the germination of a single basidiospore one year after isolation on potato-glucose agar slants.

As will be seen from this table, only one spore germinated during the first week and hyphae were visible at the point where it was inoculated. At first the hyphae were thin and arachnoid, then in a few days they developed into a dense cottony or felty mycelial mat (Fig. 1). From the second week the germination occured very erratically throughout the four months period.

White (l. c.) observed that the germination of basidiospores of the present fungus was very erratic, and when it occured, took place in distilled water or nutrient media in about 48 hours. However, the germination percentage was very small, being approximately 1.5 per cent. In the writer's experiment shown above, it was indicated that most germination occured four months after the basidiospores were put on the agar medium. This phenomenon drew the writer's attention. Accordingly, in the following experiment larger numbers of spores were observed more regularly during the longer periods of time.

Experiment (3)

In the year after the previous experiment, observation was performed upon the spore-discharge of the same fruiting-body as in June, 1950. On 25 th May, 1951 the brown basidiospores were produced just as they were in June of the previous year. They were collected and 430 single spores were isolated by the same method and technique as described before. The basidiospores which were isolated each on an agar slant were kept in the room temperature from 25 th May, 1951 to 20 th October, 1951. On 20 th October ungerminated basidiospores were taken into an incubator and kept at a constant temperature of 25°C with occasional fluctuations of ±1°C to 20th May, 1952. The number of basidiospores which were germinated during this period are shown graphically in Fig. 2.

Another 120 basidiospores each isolated in a test tube in a manner similar to the preceding ones, were observed from 1 st June, 1951 to October, 1952. They were kept throughout at room temperature. Fig. 3 shows the number of
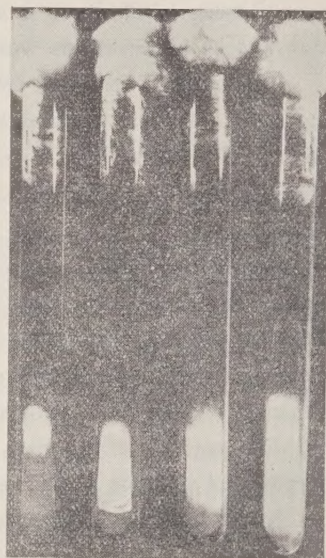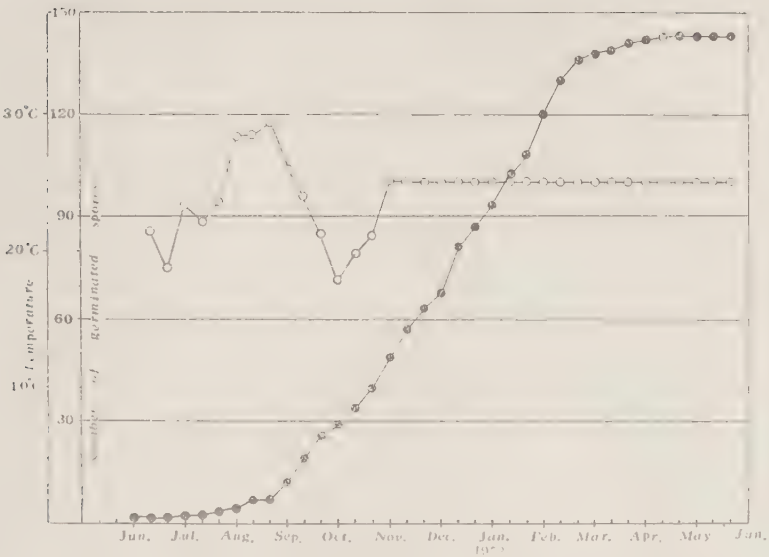
Fig. 2 Showing the numbers of germinated basidiospores from a total of 430 each isolated in an agar slant, from June, 1951 to May, 1952 kept at the room temperature for the first 4 months and at constant temperature of 25°C for the latter 8 months.
● indicates numbers of germinated basidiospores
○ indicates temperatures
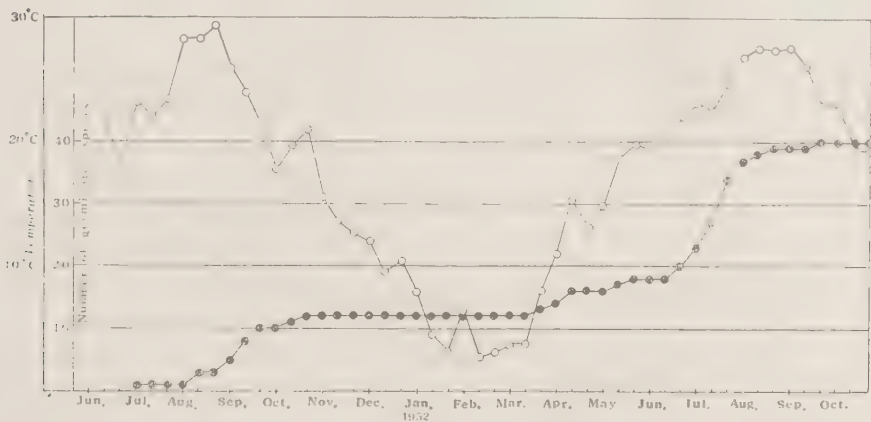
Fig. 3 Showing the numbers of germinated basidiospores from a total of 120 each isolated in an agar slant kept throughout at room temperature from June, 1951 to October, 1952.
● indicates numbers of germinated basidiospores
○ indicates temperatures

germinated basidiospores during that time.

As will be seen from Figs. 2 and 3, it is ascertained that most of the basidiospores of *Elfvingia applanata* germinate after some dormant period, and less than 0.5 per cent of the given basidiospores germinate one week after they

have been inoculated on the agar medium. White (l. c.) observed that the basidiospores of the same fungus lost their viability in about six months, if they were preserved in dry condition. It will be evident, however, that germination of the basidiospores on agar media occurs more vigorously after about six months than they do after few days. This contrasts with White's observation. His observations. so far as the writer can ascertain, were carried out during shorter periods of time and therefore it could not be discovered that the basidiospores of *Elfvingia applanata* had a long dormant time after they had been produced on the hymenium of the fruiting-body.

The germination percentage of the basidiospores during 4 summer months' period was only 7 per cent as were shown in Table 1. From Figs. 2 and 3, it was shown that the germination percentage during 5 months' period was 10 per cent. From November, however, none of the basidiospores kept at room temperature germinated owing to the low temperature which appeared to be unfavourable for the germination of basidiospores of this fungus. Owing to desiccation of the agar media, it was difficult to observe germination of basidiospores for longer periods than twelve months when kept in warmer temperatures than that of a room. As is shown by Fig. 3, some of the basidiospores on the agar medium did not germinate that year, but did begin to germinate the next spring.

Experiment (4)

Another fruiting-body of *Elfvingia applanata* was found on the stump of an oak tree (*Quercus myrsinaefolia*) about 200 meters distant from the living oak tree on which sporophores of the same fungus used in the previous experiments was grown. This sporophore first appeared on 2nd August, 1951 as a
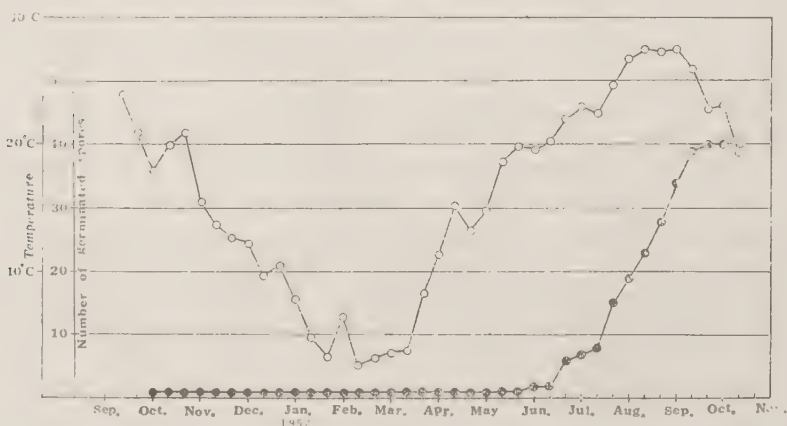
Fig. 4 Showing the numbers of germinated basidiospores from a total of 100 each isolated in an agar slant kept throughot at room temperature from Sept., 1951 to Oct., 1952.

● indicates numbers of germinated basidiospores

○ indicates temperatures

small white subglobular body. In several days it became larger and by 23 rd August the normal applanate sporophore, 15 cm long, 10 cm wide and 1.5 cm deep had developed. The fact that the tubes of the basal part of the sporophore were somewhat brownish, indicated production of basidiospores, but tubes on the outer part of the sporophore near the margin remained white and no spore discharge took place. On 25 th August, numerous brown basidiospores were produced. The writer obtained 100 basidiospores on agar slants by the same method as before. The basidiospores thus obtained were preserved at room temperature and observations were made on their germination from 10 th September, 1951 to 10 th October, 1952, at ten days intervals. The results of this observations are shown in Fig. 4.

As were shown in Fig. 4, in only one test tube the hyphae resulting from germination of a basidiospore were noticed on 1 st October, 1951 about a month after the isolation started. From 10th of September, none of the basidiospores germinated up to the next summer owing perhaps to the low temperatures which might have been unfavourable for their germinaiton. From all observations, it may be established that basidiospores of the present fungus can overwinter in a viable condition.

Experiment (5)

It was ascertained by the previous four series of experiments that basidiospores on agar media had long dormant time and that even after a year, when they were kept throughout at room temperature, they were able to germinate in a similar way to fresh spores. In this experiment, it will be determined whether basidiospores placed on a piece of paper and stored at room temperature for a year are able to germinate or not. The basidiospores used in this experiment were obtained in 1951 from the same fruiting body as were those used in the previous experiment. They were put in a Petri dish on a piece of paper and stored in a

Table 2. Difference of the germination of the fresh basidiospores and basidiospores stored at room temperature for one year.

Date examined	Basidiospores produced in 1952	Basidiospores produced in 1951
June 1, 1952	0	
10	0	
20	0	
July 1	0	
10	0	
20	1	
Aug. 1	1	
10	0	
20	2	
Sept. 1	2	
10	0	
20	3	0
Oct. 1	1	
10	1	
20	0	
May 1, 1953	3	
June 1	0	
Total	14	
Germination percentage	14%	0%

relatively cool room from 1 st June, 1951 to the next May. On 1 st June, 1952, single spore isolations were made from these basidiospores and their germination was observed from June to the end of October. At the same time fresh spores were obtained from the same fruiting body in May, 1952 and their germination was studied in the same way. One hundred single spores of each group were examined in this experiment. The results of these observations are shown in Table 2.

It will be seen from this table that the basidiospores stored for one year at room temperature in dry condition, did not germinate, while the fresh spores did germinate just as they did in the previous experiment shown just above. From the fact that the basidiospores produced in the spring, when placed on the agar medium and kept throughout at room temperature, were able to germinate in the next spring and summer, as shown in the previous experiment, it can be concluded that basidiospores on the agar medium retained their viablity, whereas those stored in a dry condition did not.

Effect of temperature upon the germination of basidiospores

Table 3. Influence of temperature upon the germination of basidiospores.

Date examined	Number of germinated basidiospores				
	20°C	23°C	25°C	28°C	33°C
November 1, 1952	0	2	2	3	2
10	1	3	1	2	0
20	1	1	1	2	0
December 1	0	2	3	3	0
10	0	1	2	2	1
20	0	1	0	1	0
January 1, 1953	0	2	2	0	0
[illegible]	[illegible]	[illegible]	0	2	0
[illegible]	[illegible]	[illegible]	1	0	0
Total	2	13	12	15	3

The methods and materials employed in this experiment were the same ones used in the previous experiments. Isolation of basidiospores on agar slants was carried out on 1st May, 1952 and they were kept at room temperature to October 20th, 1952. On 20th October, 1952, they were transferred to the incubator and kept at temperatures of 20°C, 23°C, 25°C, 28°C and 33°C, respectively. At each temperature, 50 agar slants, each with a single spore, were used. Observations were carried out for three months starting on 20th October, 1952 at ten days intervals. The results of this experiment are shown in Table 3.

From Table 3 it is shown that the basidiospores of *Elfvingia applanata* germinate from 20°C to 33°C and the optimum temperature for the germination lies between 23°C and 28°C.

Breaking the dormancy of the basidiospores by high temperature treatment

It is commonly accepted that the ascospores of some ascomycetous fungi break their dormancy by high temperature treatments. DODGE (1912) has found that heating the ascospores of *Ascobolus* to 65 to 75°C for approximately 15 minutes induced germination after they were returned to a favourable temperature. It has been also established by SHEAR and Dodge (1927) and GODDARD (1936) that the ascospores of *Neurospora* are normally dormant and will germinate only after they have been heated at the critical temperature of 49—52°C. Considering the results obtained by these investigators, some experiments were undertaken to determine whether exposure to high temperature would break the dormancy of basidiospores of the present fungus.

Experiment (1)

In this experiment the writer examined effects of high temperature treatments upon the dormancy of the basidiospores of this fungus in dry condition and in water. In dry condition, temperatures taken in the study of their effect were 50°C, 60°C and 70°C, and the times exposed were 5, 10, 30, 60 and 240 minutes, respectively. In water, temperatures were 40°C, 45°C and 50°C, and the times were 10, 30, 60 and 240 minutes. In exposing to high temperatures in dry condition, the basidiospores collected on a piece of paraffin-paper were put on a piece of cotton wool and were placed in incubators which were kept at the constant temperatures desired. In water condition, basidiospores were put in sterilized distilled water in a test tube which was kept at the constant temperatures above written in the incubators. As soon as the treatments were done, the test tubes containing the hot water suspension of basidiospores were put in water at about 10°C where cooled. The examinations of germination were carried out as were in the first experiment of the present paper.

The results of this experiment shows that among more than one thousand spores observed, none of them under any treatment germinated 48 hours after inoculation on the potato-glucose agar kept at a temperature of 25°C.

Experiment (2)

In order to observe the germination of basidiospores for longer periods of time than 48 hours, those isolated each on a potato-glucose agar slant were examined. Temperatures used in the study were 30°C, 35°C and 40°C and the times of exposure were 24 and 48 hours in each temperature range mentioned. The materials and methods in the present experiments were the same as those employed in the previous experiment. The isolations were done on 15th October, 1951 and the basidiospores thus obtained were exposed to high temperatures of 30°C, 35°C and 40°C, for 24 and 48 hours, respectively. In each temperature and time combination, 50 basidiospores were used. After the treatments all of them
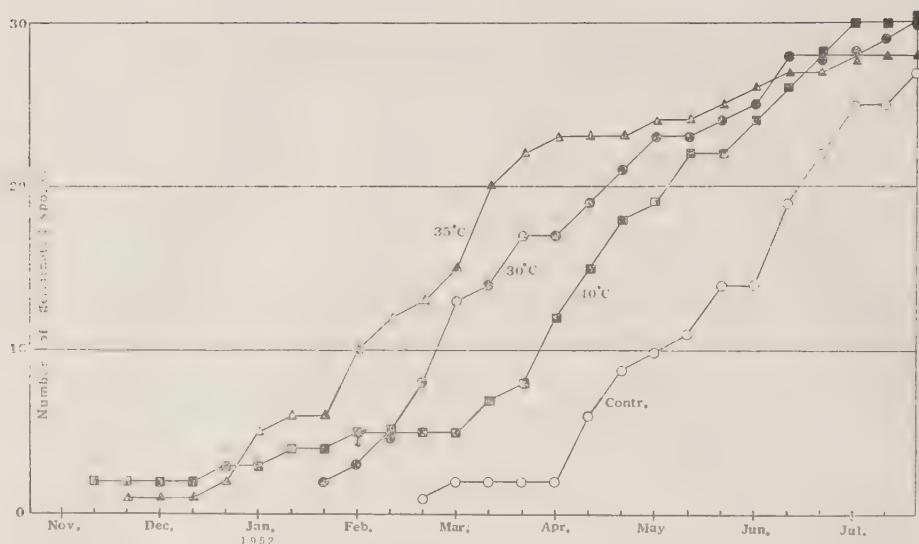
Fig. 5 Showing the numbers of germinated basidiospores from a total of 50 each isolated in an agar slant exposed to high temperatures for 48 hours.
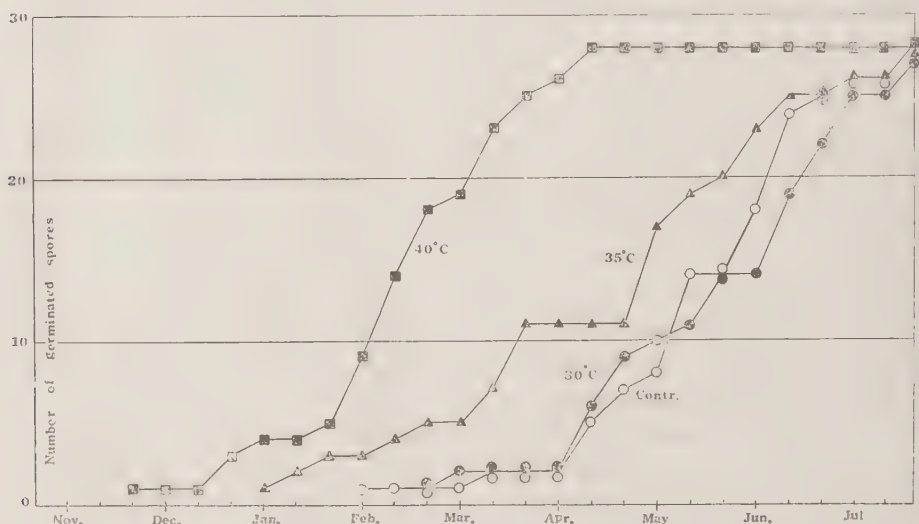
Fig. 6 Showing the numbers of germinated basidiospores from a total of 50 each isolated in an agar slant exposed to high temperatures for 24 hours.

were kept at a constant temperature range of 25—26°C. Figs. 5 and 6 show the number of germinated basidiospores exposed to the high temperatures from the beginning of November, 1951 to the end of July, 1952.

As will be seen from these figures, it appears most probable that when the basidiospores are exposed to high temperatures for 24 hours, 40°C is effective in promoting germination, 35°C is considerably effective and 30°C gives no effect at all in the course of the long periods of incubation, and that the germination

percentage at the end of the test is not influenced. In 48 hours' treatment, 35°C, 30°C and 40°C are effective in order, but significant differences do not exist between the number of spores finally germinated.

Discussion

In most of the wood-rotting Basidiomycetes, the germination of the basidiospores occurs easily on agar or in liquid media at a favourable temperature. BULLER (1909) observed germination of basidiospores of *Coriolus unicolor* and *Schizophyllum commune*, FALCK (1912) of *Gyrophanus lacrymans*, RHOADS (1918) of *Hirschioporus pargamenus*, SNELL (1922) of *Gloeophyllum saepiarium*, *G. trabeum*, *Trametes serialis*, *Fomitopsis rosea* and *Lentinus lepideus*, HIRT (1928) of *Phellinus gilvus*, MOUNCE (1929) of *Fomitopsis pinicola*, K. ITO (1942) of *Fomitopsis rhodophaea*, AOSHIMA (1950) of *Fomitopsis olivacea*, NAGAI, AOSHIMA and KOBAYASHI (1952) of *Cortinellus edodes*. Examining these data, it can be summarized that the basidiospores of any of the above species germinate easily on agar media 48 hours after inoculation at favourable temperatures.

Of the present fungus, *Elfvingia applanata*, WHITE (l. c.) observed that the germination was very erratic and when it did occur, the spores germinated 48 hours after being put on agar, in liquid media, or in distilled water. The results of his observations, thereafter, were recognized by HUBERT (1931) BAVENDAMM (1936) and CARTWRIGHT and FINDLAY (1946). In the present writer's experiments the basidiospores on various agar and in various liquid media, did not germinate within 48 hours at any temperatures tested.

By means of single spore isolations, the basidiospores on the agar slants each in a test tube, were observed on their germination for about a 12 months' period. As a result of these observations, the writer came to a conclusion that basidiospores of the present fungus had individually different dormant times ranging from one week to 12 months. The difference between the writer's and WHITE's observations is quite large, but the reason is unexplainable, without further experiments and observations. But the number of basidiospores germinated as plotted against the time, follows a normal S-shaped curve.

WHITE (l. c.) also reported that the basidiospores of this fungus lost their viability in about 6 months in dry condition. The present writer's observations, however, indicates that the basidiospores on agar slants and kept at a favourable temperature (about 25°C) germinate most vigorously after about 6 months.

Nearly ten per cent of the basidiospores produced in the fruiting-body in June in Tokyo germinated in that year, when placed on the agar medium and kept throughout at room temperature. That winter none of the basidiospores germinated but the next spring they again began to germinate, and continued to germinate until that autumn. Under these circumtances, this experiment confirms

their ability to overwinter in natural conditions.

WHITE (l. c.) reported that the germination percentage of basidiospores of this fungus was very small, about 1.5 per cent. However, if placed on agar media and kept throughout at their favourable temperature, more than 50 per cent of the spores in the present writer's experiment germinated in over 12 months.

The basidiospores of this fungus germinate at the temperature ranging from 20°C to 33°C, and the optimum temperature for the germination appears to lie between 23°C to 28°C. But it does not indicate whether or not in temperatures beyond 20°C and 33°C, they are able to germinate.

In order to break the dormancy of the basidiospores of this fungus, high temperature exposures were performed in dry condition as well as in water. Within 48 hours none of the basidiospores germinated, which is contrary to the results obtained by DODGE (l. c.), SHEAR and DODGE (1927) and GODDARD (1936) in that ascospores of *Ascobolus* and *Neurospora* were induced to germinate by high temperatures. Exposure of the basidiospores isolated on the agar media to 35°C, 30°C and 40°C for 48 hours and to 40°C and 35°C for 24 hours, did promote their germination.

In this case, however, diiference between the germination percentages in a ten months period were insignificant. Exposure of basidiospores to 30°C for 24 hours did not influence their germination at all.

In conclusion, the phenomenon of their dormancy and inducing them to break dormancy is unique in the Basidiomycetes. This may be due perhaps to the thick and special structure of their cell walls.

By the way, the writer has completed a similar experiment on the germination of basidiospores of *Ganoderma lucidum* (LEYSS) KARSTEN, and succeeded in getting clear cut results. Comparing these with the above mentioned results, it seems that the basidiospores of the species of Ganodermoideae may possibly possess the same behavior in their germination process.

Summary

In the present account the writer dealt with the germination of the basidiospores of *Elfvingia applanata* (PERS.) KARSTEN (*Fomes applanatus*).

(1). The basidiospores did not germinate on the agar or in the liquid media within 48 hours at any temperatures.

(2). The dormancy of the basidiospores were examined by the writer's experiments. Their dormant time varied from one week to 12 months according to individuals and environmental factors.

(3). More than 50 per cent of the basidiospores isolated on agar media germinated when they were observed for over the periods of 12 months.

(4). The basidiospores placed on agar media and kept throughout at room

temperature germinated considerably after nearly one year, while none of the basidiospores germinated which were stored in a dry condition for a corresponding period of time at room temperature.

(5). The basidiospores germinate at temperatures ranging from 20 to 33°C and the optimum temperature for germination appeared to lie between 23°C to 28°C.

(6). Exposure of the basidiospores isolated on agar slants to 35°C for 48 hours and to 40°C for 24 hours resulted in earlier germination but did not increase final germination.

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